Acta Poloniae Pharmaceutica – Drug Research, Vol. 59 No. 2 pp. 139–143, 2002

ISSN 0001-6837
Polish Pharmaceutical Society

FLAVONOIDS FROM LEMON BALM (*MELISSA OFFICINALIS* L., *LAMIACEAE*)

JOLANTA PATORA and BARBARA KLIMEK

Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Łódź, Poland

Abstract: Six flavonoids have been isolated from the leaves of lemon balm (*Melissa officinalis* L., *Lamiaceae*). Their structures were determined on the basis of spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR and FAB MS) as luteolin, luteolin 7–O–β–D–glucopyranoside, apigenin 7–O–β–D–glucopyranoside, luteolin 7–O–β–D–glucuronopyranoside, luteolin 3'–O–β–D–glucuronopyranoside and luteolin 7–O–β–D–glucopyranoside–3'–O–β–D–glucuronopyranoside. The last three glycosides have been found in lemon balm for the first time and luteolin 7–O–β–D–glucopyranoside–3'–O–β–D–glucuronopyranoside is a new compound found in plants.

Keywords: *Melissa officinalis* L., lemon balm, flavonoids, luteolin glucuronides

Melissa officinalis L. (*Lamiaceae*) – lemon balm is known as a medicinal plant for *ca* 2000 years. The plant grows on natural places in mediterranean areas and now it is cultivated or naturalized all over Europe. *Melissae folium* (FP V, DAB 10, Ph. Eur. Suppl. 2000) is used as sedative, antispasmodic and also antiviral remedy (1–4). Over 300 different phytopharmaceutical preparations containing *Melissae folium* or extracts from it are produced in Europe (2). Antiinflammatory (5), gonadotropic (2), thyreotropic (2;6) and cholinergic (7) activities are also attributed to the plant (7). Some of the activities may be connected with the phenolic compounds occurring in lemon balm which include rosmarinic acid, *Labiatae* tannins and flavonoids. Among flavonoids apigenin 7–O–glucoside and luteolin 7–O–glucoside as well as three flavonols: rhamnocitrin, rhamnazin and isoquercitrin have previously been reported (8;9). Recently, we have informed on some other flavonoids found in *Melissae folium* (10;11) and the present paper contains a detailed description of their isolation and structural determination.

EXPERIMENTAL

Equipment and methods

Melting points were determined on Boetius, uncorrected. UV spectra with shift reagents were made according to the standard procedure (12) on Unicam SP–800, IR on Specord M–80, ^{1}H and ^{13}C NMR on Bruker 500 MHz in DMSO–d_6 and FAB MS on Finnigan Mat 95 with Cs^+ ions at 13 KeV.

Total acid hydrolysis: 10% H_2SO_4, 100°C, 3h.

Partial acid hydrolysis: 1N CF_3COOH, 100°C, 15 min.

Column chromatography (CC) was performed on glass columns with the use of the following materials: polyamide (Roth), cellulose (S&S), silica gel (MN Kieselgel 60 70–270 mesh), Sephadex LH–20 (Pharmacia); thin–layer chromatography (TLC) on silica gel (Merck) and cellulose (Merck), paper chromatography (PC) on Whatman No. 1 and No. 3.

The following solvent systems were employed to TLC and PC:

S_1 – n–BuOH / CH_3COOH / H_2O 4:1:5 (upper layer)
S_2 – CH_3COOH / H_2O 15:85
S_3 – EtOAc / HCOOH / H_2O 18:1:1
S_4 – $CHCl_3$ / MeOH / H_2O 6:3:1 (lower layer)
S_5 – CH_3COOH / H_2O 6:4
S_6 – $CHCl_3$ / C_6H_6 / MeOH 3:2:0.5
S_7 – n–BuOH / pyridine / H_2O 6:4:3
S_8 – EtOH 96° / NH_4OH 25% / H_2O 20:1:4

Flavonoids were visualized by UV light 366 nm, NH_3 fumes and 1% $AlCl_3$ in MeOH. Sugars were detected with aniline phthalate solution in n–butanol.

Plant material

The leaves of *Melissa officinalis* L. subsp. *officinalis* were collected from plants cultivated in Gostyń before flowering time in July 1999 and dried. The identification of the plant material was performed on the basis of morphological and anatomical features.

Extraction and isolation

Powdered plant material (800 g) was pre–extracted with petrol followed by $CHCl_3$ in Soxhlet. Then, it was extracted successively with boiling

MeOH and MeOH 70° to obtain extracts EMe and EMeW, respectively. EMe was evaporated, dissolved in water and partitioned between ethyl ether, ethyl acetate to obtain extracts EEt (12 g) and EEtAc (20 g) respectively. EMeW was evaporated separately, dissolved in H_2O and extracted with n–BuOH until exhausted. After removing the solvent EBu (25 g) was obtained.

Isolation of luteolin (I)

EEt was submitted to column chromatography on silica gel using $CHCl_3$ / C_6H_6 / MeOH (3:2:0.5) as the eluent. Fractions 13–16 were combined, evaporated under reduced pressure, dissolved in MeOH and purified on a Sephadex LH–20 column. From the dry residue of the flavonoid fractions compound **I** (15 mg) was obtained by crystallization from MeOH.

Isolation of apigenin 7–O–β–D–glucoside (II) and luteolin 7–O–β–D–glucoside (III)

Extract EEtAc was submitted to column chromatography using first, cellulose with a mixture of EtOAc / MeOH / H_2O (200:33:27) as the eluent and then, silica gel with the solvent system $CHCl_3$ / MeOH / H_2O (6:3:1) as the eluent. Fractions 24–32 from the second column yield compound **II** (15 mg) and from the fractions 35–40 compound **III** (20 mg) was obtained.

Isolation of luteolin glucuronides (IV–VI)

The isolation of glucuronides from the butanolic extract (EBu) was performed by CC using first polyamide followed by silica gel. Water and increasing gradient of MeOH in H_2O were used as eluents for CC on the polyamide and solvent system EtOAc / MeOH / H_2O (200:33:27) followed by $CHCl_3$ / MeOH / H_2O (64:36:8) for CC on silica gel. The combined fractions 10–147 from the polyamide column were rechromatographed using the silica gel column and preparative PC to yield compound **IV** (10 mg). From fractions 148–196 (the polyamide column), compound **V** (15 mg) was obtained by the use of a similar method. The combined fractions 197–212 from the polyamide column were evaporated to dryness and from the residue compound **VI** was separated by crystallization from MeOH 70°.

Identification of the isolated compounds

Luteolin (I)

m.p. 325–328°C (13); R_f: S_1 – 0.79; S_2 – 0.05; S_3 – 0.90; S_4 – 0.88; S_5 – 0.45; S_6 – 0.08.

$IR_{\nu\ max}^{KBr}$ cm^{-1}: 3490–3400 (OH), 2923–2617 (C–H), 1655 (C=O in flavon), 1610–1490 (aromatic rings), 1458, 1364, 1267.

$UV_{\lambda\ max}^{MeOH}$ nm: 253, 268, 290sh, 348; + NaOMe: 265, 330sh, 402; + $AlCl_3$: 274, 300sh, 329sh, 425; + $AlCl_3$/HCl: 275, 296, 356, 388; + NaOAc: 271, 325sh, 390; + NaOAc/H_3BO_3: 262, 301sh, 372, 430sh.

^1H NMR; δ ppm: 7.40 (dd, J=2.1Hz; 8.3 Hz, H–6'); 7.38 (d, J=2.2 Hz, H–2'); 6.87 (d, J=8.2 Hz, H–5'); 6.65 (s, H–3); 6.43 (d, J=2 Hz, H–8,); 6.18 (d, J=2 Hz, H–6).

Apigenin 7–O–β–D–glucopyranoside (II)

m.p. 223–226°C (13); R_f values S_1 – 0.55; S_2 – 0.20; S_3 – 0.37; S_4 – 0.43 in accordance with the authentic sample.

IR, UV, and ^1H NMR spectral data are in accordance with the literature data (9).

Luteolin 7–O–β–D–glucopyranoside (III)

m.p. 248–250°C (13), R_f: S_1 – 0.34; S_2 – 0.12; S_3 – 0.30; S_4 – 0.31 identical as for the authentic sample.

IR, UV, and ^1H NMR spectral data are in accordance with the literature data (9).

Luteolin 7–O–β–D–glucopyranoside–3'O–β–D–glucuronopyranoside (IV)

m.p. 196–203°C; R_f: S_1 – 0.13; S_2 – 0.30; S_3 – 0.03; S_4 – 0.02.

$IR_{\nu\ max}^{KBr}$ cm^{-1}: 3500–3300 (OH), 2930–2853 (C–H), 1710 (COOH), 1635 (C=O in flavon), 1604–1498 (aromatic rings), 1456, 1417, 1377, 1282.

$UV_{\lambda\ max}^{MeOH}$ nm: 239sh, 270, 345; + NaOMe: 255, 327sh, 396; + $AlCl_3$: 278, 298sh, 345, 384; + $AlCl_3$/HCl: 255sh, 282, 298, 345, 384; + NaOAc: 228, 270, 394; + NaOAc/H_3BO_3: 231, 269, 343.

^1H NMR; δ ppm: 7.70 (2H, dd, J=2.3 Hz and 6.0 Hz; H–2' and H–6'); 6.90 (d, J=8,0 Hz, H–5'); 6.78 (d, J=2.2 Hz, H–8); 6.72 (s, H–3); 6.40 (d, J=2.1 Hz, H–6); 5.30 (d, J=7.6 Hz, H–1'' gluc.); 4.83 (d, J=6.2 Hz, H–1'''glucur.acid); 3.85 (dd, J=1.5 Hz and 11.2 Hz, H–6''–gluc.); 3.70 (d, J=10.2 Hz, H–5'''); 3.19–3.35 (seven sugar protons).

FAB MS negative ion mode *m/z*: 623.3 $(M)^-$, 459.3 $(M–gluc.–2H)^-$; positive ion mode *m/z*: 447.3 $(M–glucuronic\ acid)^+$, 419.2 $(M–gluc.–CO_2)^+$.

Products of total acid hydrolysis: luteolin (R_f: S_1 – 0.79; S_2 – 0.05; S_5 – 0.45), glucose (R_f: S_1 – 0.15; S_7 – 0.35; S_8 – 0.41), glucuronic acid and its lactone (R_f: S_1 – 0.10 and 0.30 ; S_7 – 0.15 and 0.61; S_8 – 0.34 and 0.55).

Products of partial acid hydrolysis: glucose and luteolin 3'–O–glucuronopyranoside (compound **VI**), identified by PC and TLC in comparison to the authentic samples.

Luteolin 7–O–β–D–glucuronopyranoside (V)

m.p. 210–215°C; R_f: S_1 – 0.26; S_2 – 0.13; S_3 – 0.19; S_4 – 0.10 in accordance with the authentic substance (15).

$IR_{\nu\ max}^{KBr}$ cm^{-1}: 3480–3300 (OH), 2923–2853 (C–H), 1710 (COOH); 1655 (C=O in flavon), 1607–1498 (aromatic rings), 1456, 1374, 1259.

$UV_{\lambda\ max}^{MeOH}$ nm: 256, 267, 349; + NaOMe: 265, 303sh, 402; + $AlCl_3$: 275, 300sh, 425; + $AlCl_3$/HCl: 270, 298sh, 362, 390; + NaOAc: 268, 408; + NaOAc/H_3BO_3: 265, 372.

^1H NMR; δ ppm: 7.43 (d, J=2 Hz, H–6'); 7.41 (d, J=2 Hz, H–2'); 6.88 (d, J=8.4 Hz, H–5'); 6.77 (d, J=2 Hz, H–8,); 6.73 (s, H–3); 6.42 (d, J=2 Hz, H–6); 5.11 (d, J=7.4 Hz, H–1''); 3.68 (d, J=9.5 Hz, H–5''); 3.21–3.68 (m, H–2'', H–3'', H–4'').

Total acid hydrolysis gave luteolin (R_f: S_1 – 0.79; S_2 – 0.05; S_5 – 0.45), glucuronic acid and its lactone (R_f: S_1 – 0.10 and 0.30; S_7 – 0.15 and 0.61; S_8 – 0.34 and 0.55).

Luteolin 3'–O–β–D–glucuronopyranoside (VI)

m.p. 199–205°C (16); R_f: S_1 – 0.39 ; S_2 – 0.13; S_3 – 0.24; S_4 – 0.17.

$IR_{\nu\ max}^{KBr}$ cm^{-1}: 3480–3246 (OH), 2929–2605 (C–H), 1701 (COOH), 1657 (C=O in flavon), 1605–1490 (aromatic rings), 1438, 1398, 1366, 1285, 1262.

$UV_{\lambda\ max}^{MeOH}$ nm: 240, 270, 343; + NaOMe: 256, 327, 394; + $AlCl_3$: 278, 298, 345, 382sh; + $AlCl_3$/HCl: 255, 280, 298, 345, 382; + NaOAc: 229, 274, 390; + NaOAc/H_3BO_3: 232 , 271, 345.

^1H NMR; δ ppm: 7.68 (d, J=2 Hz; H–2'); 7.64 (dd, J=2 Hz, 8.6 Hz, H–6'); 6.98 (d, J=8.5 Hz, H–5'); 6.73 (s, H–3), 6.49 (d, J=2.1 Hz, H–8,); 6.20 (d, J=2 Hz, H–6); 5.21 (d, J=7 Hz, H–1''); 4.05 (d, J=9,5 Hz, H–5''); 3.38–3.47 (m, H–2'', H–3'', H–4'').

FAB MS (negative ion mode) *m/z*: 461 $(M)^-$, 285 $(aglycone)^-$.

^{13}C NMR; δ ppm: 163.32 (C–2); 103.29 (C–3); 181.83 (C–4); 161.43 (C–5); 98.88 (C–6); 164.22 (C–7); 94.12 (C–8); 157.35 (C–9); 103.75 (C–10); 121.93 (C–1'); 113.78 (C–2'); 145.15 (C–3'); 150.76 (C–4'); 116.64 (C–5'); 121.60 (C–6'); 100.62 (C–1''); 71.38 (C–2''); 72.97 (C–3''); 75.42 (C–4'', C–5''); 170.18 (C–6'').

Total acid hydrolysis gave luteolin (R_f: S_1 – 0.79; S_2 – 0.05; S_5 – 0.45), glucuronic acid and its lactone (R_f: S_1 – 0.10 and 0.30; S_7 – 0.15 and 0.61; S_8 – 0.34 and 0.55).

RESULTS AND DISCUSSION

Methanolic and hydromethanolic extracts from *M. officinalis* leaves were partitioned between diethyl ether, ethyl acetate and n–butanol.

From the ether extract compound **I** was isolated by column chromatography on silica gel and Sephadex. Compound **I** was identified as luteolin (5, 7, 3', 4'– tetrahydroxyflavon) on the basis of IR, UV and ^1H NMR spectral analysis, melting point and R_f values, which were identifical with obtained for authentic sample. Free flavonols: rhamnocitrin and ramnazin, reported previously from *M. officinalis* L. (8;9), have not been found among flavonoids isolated by us from the investigated plant material. Luteolin (in aglycone form) has not been listed as a constituent of *M. officinalis* L. yet.

From the ethyl acetate extract compounds **II** and **III** were isolated and identified (UV, IR, ^1H NMR) as apigenin 7–O–glucoside and luteolin 7–O–glucoside, respectively. Both compounds have already been described as the constituents of *M. officinalis* by other autors (9). The flavonol glycoside isoquercetin, reported previously in lemon balm (9), has not been found by us.

Further three flavonoid glycosides (compounds **IV**–**VI**) were isolated from the butanolic extract. Compound **IV** (Figure 1) liberated luteolin and two sugar components as the products of the total acid hydrolysis. The sugars have been identified (PC, TLC) as glucose and glucuronic acid. Partial acid hydrolysis gave two monoglycosides. One of them was identical with compound **VI** and the second with luteolin 7–O–glucoside (**III**). The

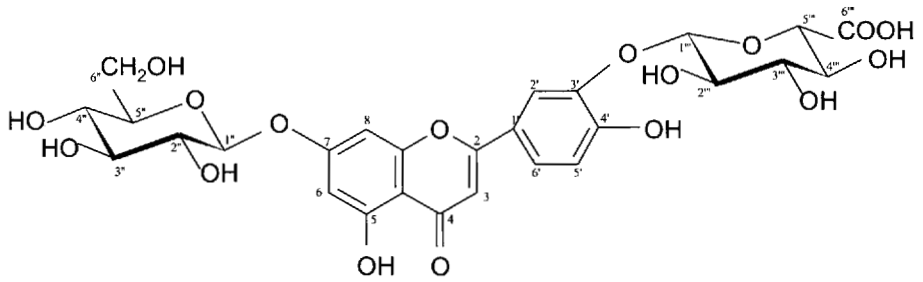

Figure 1. Luteolin 7–O–β–D–glucopyranoside–3'–O–β–D–glucuronopyranoside (**IV**)

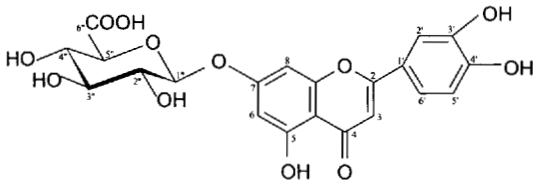

Figure 2. Luteolin 7–O–ß–D–glucuronopyranoside (**V**)

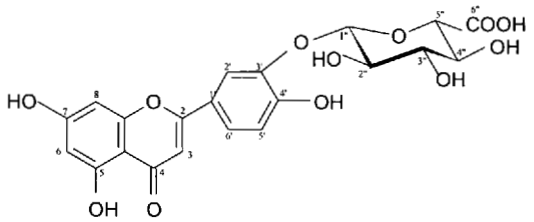

Figure 3. Luteolin 3'–O–ß–D–glucuronopyranoside (**VI**)

UV spectral analysis with the use shift reagents (12) showed the presence of free 4'–hydroxyl group (the effect of NaOMe), as well as the lack of free hydroxyl group at C–7 (UV spectrum with NaOAc) and at C–3' (the UV spectrum with NaOAc/H_3BO_3, $AlCl_3$ and $AlCl_3$/HCl) in flavon structure, for details see Experimental. ^{1}H NMR spectrum of **IV** revealed in aromatic region proton signals characteristic for luteolin and in aliphatic region two characteristic doublets at 5.30 (J=7.6 Hz) and 4.83 (J=6.2 Hz), which could be attributed to the anomeric protons of glucose and glucuronic acid, respectively as well as a doublet at 3.70 (J=10.2 Hz) attributed to H–5 of glucuronic acid (14). In IR spectrum the presence of glucuronic acid in **IV** has been demonstrated by a signal of carboxyl group (1710 cm^{-1}). FAB MS of **IV** exhibited a quasimolecular ion at *m/z* 623.3 (negative ion experiment) and characteristic fragment ion at 447.3 (positive ion experiment) resulting from the detachment of glucuronic acid moiety. The mass spectral data pointed that **IV** is a diglycoside consisting of luteolin, glucose and glucuronic acid, one molecule each. Considering the UV spectral data, ^{1}H NMR and FAB MS, the structure of **IV** could be determined as luteolin 7–O–β–D–glucopyranoside–3'–O–β–D–glucuronopyranoside. It is a new structure isolated from plant kingdom.

Compound **V** (Figure 2) was identified as luteolin 7–O–β–D–glucuronopyranoside by comparison (R_f, IR, UV, ^{1}H NMR) with the authentic sample (15). It has been found in *M. officinalis* L. for the first time.

Compound **VI** (Figure 3) gave the same products of hydrolysis as compound **V**, namely, luteolin and glucuronic acid. The UV spectral analysis showed a free hydroxyl group at C–7 in luteolin and the lack of ortho–dihydroxy group in ring B as well as a free OH at C–4'. These findings suggested that sugar moiety is attached to OH at the C–3' position. ^{1}H and ^{13}C NMR as well as FAB MS spectral data confirmed that compound **VI** is luteolin 3'–O–β–D–glucuronopyranoside. Recently, the same structure has been described from *Rosmarinus officinalis* L. (16) and *Salvia officinalis* L. (17). In the *M. officinalis* leaves compound **VI** is the main constituent of the flavonoid fraction.

Luteolin glucuronides are well soluble in water and also in methanol 70°. They can be easily extracted from plant material with the solvents which are used to prepare infusion, decoction or tincture. After internal use it is hydrolyzed by β–glucuronidase to luteolin (18), which possesses vasorelaxant (19), antioxidative, antiinflammatory and antiallergic activities (20). Therefore, besides volatile terpenes, flavonoid compounds may also influence therapeutic effect of lemon balm.

Acknowledgement

This work was supported by grant No. 502–13–753 (190) from the Medical University of Łódź.

REFERENCES

1. Soulimani R., Fleurentin J., Mortier F., Misslin R., Derrieu G., Pelt J.M.: Planta Med. 57, 105 (1991).
2. Schultze W., Zanglein A., Klose R., Kubeczka K.H.: Deutsch. Apoth. Ztg. 129, 155 (1989).
3. Koytchev R., Alken R.G., Dundarov S.: Phytomedicine 6, 225 (1999).
4. Yamasaki K., Nakano M., Kawahata T., Mori H., Otake T., Ueba N., Oishi I., Inami R., Yamane M., Nakamura M., Murata H., Nakanishi T.: Biol. Pharm. Bull. 21, 829 (1998).
5. Howes M.J.R., Houghton P.J., Hoult J.R.S.: Int. Congress and 48th Annual Meeting of Society for Medicinal Plant Research, Abstract SL 16, Zurich 2000.
6. Auf'mkolk M., Ingbar J.C., Kubota K., Amir S.M., Ingbar S.H.: Endocrinology 116, 1687 (1985).
7. Perry E.K., Pickering A.T., Wang W.W., Houghton P.J., Perry N.S.: J. Pharm. Pharmacol 51, 527 (1999).
8. Thieme H., Kitze C.: Pharmazie 28, 69 (1973).

9. Mulkens A., Kapetanidis I.: Pharm. Acta Helv. 62, 19 (1987).
10. Patora J., Klimek B.: 2000 Years of Natural Product Research – Joint Meeting of the ASP, AFERP, GA and PSE, Abstract No. 601, Amsterdam, July 26–30 1999.
11. Patora J., Klimek B.: The 3rd Conference: Flavonoids and their use, pp. 15–19, Rzeszów, May 2000.
12. Mabry T.J., Markham K.R., Thomas M.B.: The systematic identification of flavonoids, Springer, Berlin–Heidelberg–New York 1970
13. Karrer W.: Konstitution und Vorkommen der organischen Pflanzenstoffe (exclusive Alkaloide), Birkhauser Verlag Basel und Stuttgart, 1976.
14. Harborne J.B. (Ed.): The Flavonoids: Advances in research since 1986, Champan–Hall, London 1993.
15. Klimek B.: Acta Polon. Pharm. 52, 53 (1995).
16. Okamura N., Haraguchi H., Hashimoto K., Yagi A.: Phytochemistry 37, 1463 (1994).
17. Lu Y., Foo L.Y.: Phytochemistry 55, 263 (2000).
18. Shimoi K., Saka N., Kaji K., Nozawa R., Kinae N.: Biofactors 12, 181 (2000).
19. Sanchez de Rojas V.R., Somoza B., Ortega T., Villar A.M.: Planta Med. 62, 554 (1996).
20. Kimata M., Inagaki N, Nagai H.: Planta Med. 66, 25 (2000).

Received: 9.10.2001